Organizing Organoids

Organizing organoids: stem cells branch out

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Summary:
In this issue, Taguchi and Nishinakamura (2017) describe a carefully optimized method for making a branch-competent ureteric bud, a tissue fundamental to kidney development, from mouse embryonic stem cells and human induced pluripotent stem cells. The work illuminates embryology and has important implications for making more realistic kidney organoids.

Main article:
Stem cells are particularly promising for the production of human induced pluripotent stem cell (hiPSC)-derived organoids (Bartfeld and Clevers, 2017). These multicellular entities allow biomedical mechanisms to be directly explored in human systems and, in the future, organoids may also be the basis of transplant therapies. Although most organoids have realistic micro-scale anatomies, they lack large-scale organization characteristic of true organs. In this issue, Taguchi and Nishinakamura take an important step towards addressing this discrepancy in hiPSC-derived renal organoids (Taguchi & Nishinakamura, 2017).

Kidneys develop mainly from three types of renogenic stem cells: nephron progenitor (NP) mesenchymal cells, which make epithelial excretory nephrons, stromal progenitor (SP) mesenchyme cells, which make supportive stroma, and ureteric progenitor (UP) cells, which make the ureter and collecting duct tree. Attempts to engineer renal organoids from stem cells began with crude reaggregations of NP, SP and UP cells obtained directly from mouse embryos. The result of reaggregating suspensions containing these three cell types was the formation of nephrons, stroma, and multiple collecting duct 'treelets' (Unbekandt & Davies, 2010). Each component was realistic, but they were arranged in a messy jumble rather than being organized around the framework of a single collecting duct tree. Why the mess? In the embryo, the UP cells enter an area of mixed NP and SP cells as an intact epithelial tubule, called the ureteric bud, which branches to provide the framework around which other renal structures are arranged. In crude reaggregates, UP cells are scattered and the coherent organizing framework is missing. A framework can be imposed artificially by a technique in which just one collecting duct from a reaggregate is isolated and placed in a new reaggregate of fetal-derived NP and SP cells. This forced organization results in a much

more realistic organoid, with nephrons arranged around and connecting to a single collecting duct tree, as well as proper cortico-medullary organization (Ganeva et al., 2011; Chang & Davies 2012).

These techniques cannot be transferred directly to human cells, because both ethical and practical reasons restrict the availability of appropriate ex-fetu material. Generating human renal organoids therefore depends on manipulation of human induced pluripotent stem cells (hiPSCs). Takasato et al. (2015) previously developed an efficient method for differentiating human iPS cells into mixed NP, SP and UP-like cells. When cultured, they produced realistic nephrons and stroma, as well as immature ureteric-bud like tubules. These ureteric tubules, however, showed only modest branching and the presence of multiple scattered UP tubules meant there was no realistic large-scale organization.

Given the dramatic improvement achievable in the ex-fetu mouse system through the combination of UP cells in a single tubule with pure fetal NP and SCs, Taguchi and Nishinakumura (2017)focused on the goal of making fully branch-capable UP tubules from hiPSCs. Very sensibly, they began not with hiPS cells but with mouse embryos, which they used to analyse natural step-wise development of the ureteric bud. Importantly, they worked backwards, from recapitulating the last stages of bud maturation from nearly-mature buds, to understanding and recapitulating earlier and earlier stages. In this way, they were always moving from an experimental early step to a known and proper sequence of later steps, markedly increasing their chances of success. At each step, their choice of signalling molecules to guide differentiation in culture was based on analysis of the receptors expressed by the cells and the authors' previous discovery that UPs come from more anterior parts of the embryo than do NPs (Taguchi et al., 2014). The result of this painstaking work was an efficient method to convert mouse embryonic stem cells (mESCs) into UP cells that formed branching tubules in 3D gels. When placed in culture with mixed ex-fetu mouse NP and SP cells, these UP cells also branched to organize an anatomically realistic kidney organoid. Encouragingly, the system also worked when mESC-derived UBs were combined with mESC-derived NPs, although development was poor unless ex-fetu SPs were also provided (there is not yet a method for making SPs from mESCs). The authors went on to apply their protocol to human iPS (hiPS) cells, with a few necessary changes of timing. The hiPSC-derived UP formed a branching tubule tree in 3D gels and, when placed in hiPS-derived NP cells during a pilot experiment, they showed some branching and organizing activity. Presumably, as in the mouse experiments, addition of SPs would have greatly improved organoid development: human SPs are not, however, available.

A method to create branch-competent UPs from hiPS cells is an important step towards generating

more realistic human organoids. The next steps will be to develop methods to derive SPs from hiPSCs that will be available to mix with hiPS-derived NPs. In principle, if an hiPSC-derived UP tubule can be surrounded by hiPSC-derived SPs and NPs, hiPSCs might generate an entire and properly organized kidney organoid. There may even be scope to apply a technique recently developed in the mouse system, in which asymmetric BMP signalling environments can direct the development of one branch of a collecting duct tree into a uroplakin-positive ureter while other branches serve to organize a kidney (Mills et al., 2017). If this technique is feasible, hiPSC organoids will have a urine drain. Moreover, efforts to attract host vascularization within transplanted hiPSC-derived organoids, similarly to vascularization seen in transplanted mouse renal organoids (Xinaris et al., 2012), may provide opportunities to perform functional studies in transplants within the host animal.

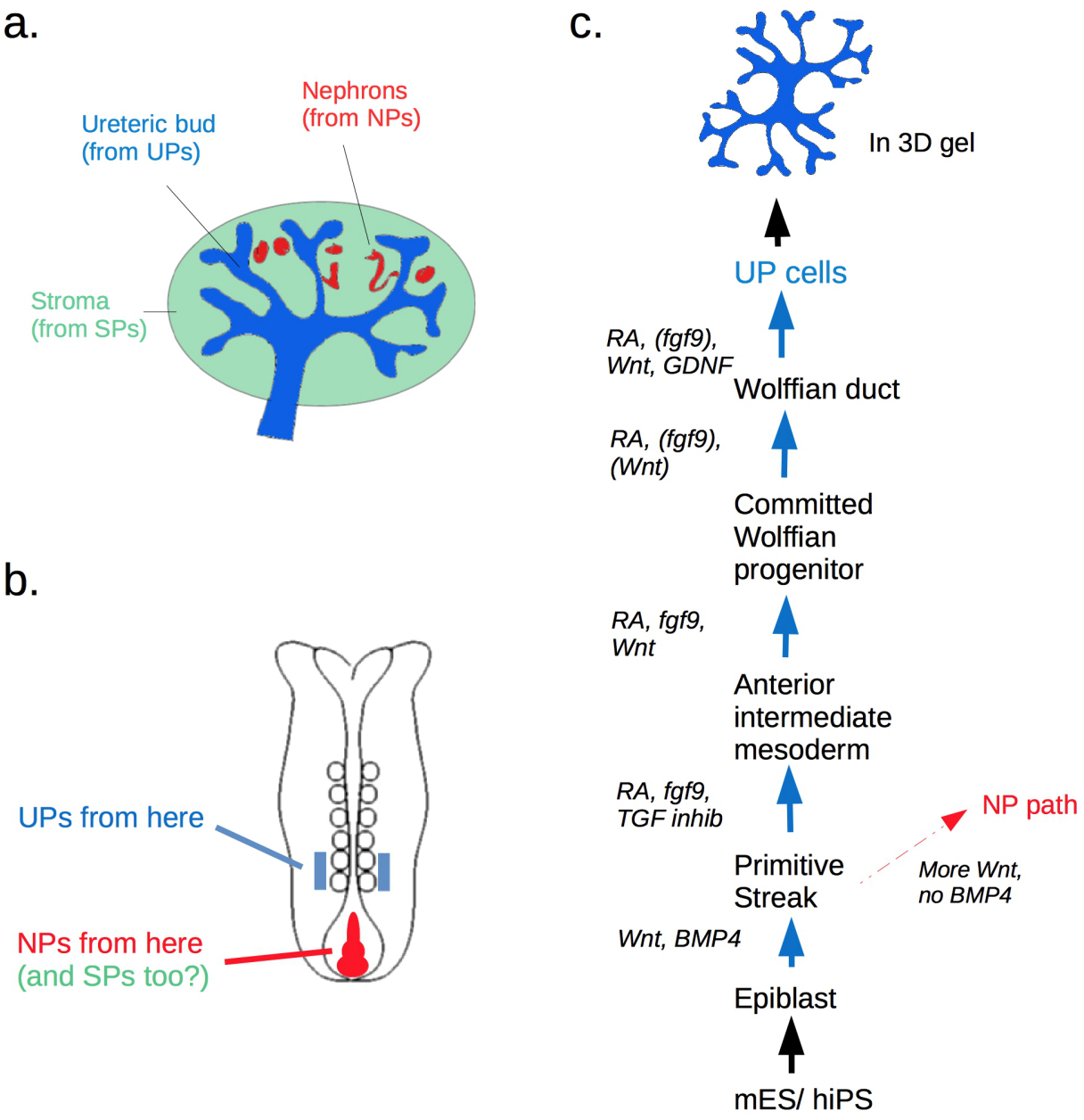

Figure 1: Generating ureteric bud progenitor (UP) cells. (a) Depiction of s a developing kidney and the contribution from UP cells, nephron progenitor (NP) cells, and stromal progenitor (SP) cells. (b) Depiction of the different antero-posterior levels of the intermediate mesoderm of the embryo that give rise to UP and NP cells, as discovered by Taguchi et al. in 2014. (c) Depiction, reading downwards, of the order in which Taguchi & Nishinakamura analysed UP development, and reading upwards, the method for producing UP cells from mESCs, and with minor differences, hiPSCs.

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